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**Title:** THE INSPIRED SINEWAVE TECHNIQUE: A NOVEL METHOD TO MEASURE LUNG VOLUME AND VENTILATORY HETEROGENEITY

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**Author Conflict:** No competing interests declared

**Running Title:** The inspired sinewave technique: a novel test of lung function

**Abstract:** The Inspired Sinewave Technique (IST) is a novel method which can provide simple, non-invasive cardiopulmonary measurements. Over successive tidal-breaths the concentration of a tracer gas (i.e. nitrous oxide, N<sub>2</sub>O) is sinusoidally modulated in inspired air. Using a single-compartment tidal-ventilation lung model, the resulting amplitude/phase of the expired sinewave allows estimation of end-expired lung volume (ELV), pulmonary blood flow and three indices for ventilatory heterogeneity (VH; ELV<sub>180</sub>/FRC<sub>pleth</sub>, ELV<sub>180</sub>/FRC<sub>pred</sub> and ELV<sub>60</sub>/ELV<sub>180</sub>). This investigation aimed to determine: the repeatability and agreement of ELV with FRC<sub>pleth</sub>, and, as normal ageing results in well-established changes in pulmonary structure and function, whether the IST estimates of ELV and VH are age dependent. 48 healthy never-smoker participants (20-

86 years) underwent traditional pulmonary function testing: (e.g. spirometry, body plethysmography) and the IST test which consisted of 4 minutes of quiet breathing through a facemask while inspired N<sub>2</sub>O concentrations are oscillated in a sinewave pattern with a fixed mean (4%) and amplitude (3%) and a period of either 180 seconds or 60 seconds.  $ELV^{180}/FRC^{pleth}$  and  $ELV^{180}/FRC_{pred}$  were age dependent (average decreases of 0.58% and 0.48% per year) suggesting an increase in VH with advancing age. ELV showed a mean bias of -1.09L vs.  $FRC_{pleth}$ , but when normalised for the effects of age this bias reduced to -0.35L. The IST test has potential to provide clinically useful information, (e.g. for mechanically ventilated patients), but these findings suggest that the increases in VH with healthy ageing must be accounted for in clinical investigations.

**New Findings:** We present a new non-invasive medical technology - the inspired sinewave technique - which involves inhaling sinusoidally fluctuating concentrations of a tracer gas. The technique requires only passive patient cooperation, and can monitor different cardiorespiratory variables such as end expired lung volume, ventilatory heterogeneity and pulmonary blood flow. In this article we demonstrate that the measurements of end expired lung volume are repeatable and accurate, in comparison to whole body plethysmography, and the technique is sensitive to the changes in ventilatory heterogeneity associated advancing ageing. As such, it has the potential to provide clinically valuable information.

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# **THE INSPIRED SINEWAVE TECHNIQUE: A NOVEL METHOD TO MEASURE LUNG VOLUME AND VENTILATORY HETEROGENEITY**

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## **NEW FINDINGS**

We present a new non-invasive medical technology - the inspired sinewave technique - which involves inhaling sinusoidally fluctuating concentrations of a tracer gas. The technique requires only passive patient cooperation, and can monitor different cardiorespiratory variables such as end expired lung volume, ventilatory heterogeneity and pulmonary blood flow.

In this article we demonstrate that the measurements of end expired lung volume are repeatable and accurate, in comparison to whole body plethysmography, and the technique is sensitive to the changes in ventilatory heterogeneity associated advancing ageing. As such, it has the potential to provide clinically valuable information.

## ABSTRACT

The Inspired Sinewave Technique (IST) is a novel method which can provide simple, non-invasive cardiopulmonary measurements. Over successive tidal-breaths the concentration of a tracer gas (i.e. nitrous oxide,  $N_2O$ ) is sinusoidally modulated in inspired air. Using a single-compartment tidal-ventilation lung model, the resulting amplitude/phase of the expired sinewave allows estimation of end-expired lung volume (ELV), pulmonary blood flow and three indices for ventilatory heterogeneity (VH;  $ELV_{180}/FRC_{pleth}$ ,  $ELV_{180}/FRC_{pred}$  and  $ELV_{60}/ELV_{180}$ ).

This investigation aimed to determine: the repeatability and agreement of ELV with  $FRC_{pleth}$ , and, as normal ageing results in well-established changes in pulmonary structure and function, whether the IST estimates of ELV and VH are age dependent.

48 healthy never-smoker participants (20-86 years) underwent traditional pulmonary function testing: (e.g. spirometry, body plethysmography) and the IST test which consisted of 4 minutes of quiet breathing through a facemask while inspired  $N_2O$  concentrations are oscillated in a sinewave pattern with a fixed mean (4%) and amplitude (3%) and a period of either 180 seconds or 60 seconds.

$ELV_{180}/FRC_{pleth}$  and  $ELV_{180}/FRC_{pred}$  were age dependent (average decreases of 0.58% and 0.48% per year) suggesting an increase in VH with advancing age. ELV showed a mean bias of -1.09L vs.  $FRC_{pleth}$ , but when normalised for the effects of age this bias reduced to -0.35L. The IST test has potential to provide clinically useful information, (e.g. for mechanically ventilated patients), but these findings suggest that the increases in VH with healthy ageing must be accounted for in clinical investigations.

## INTRODUCTION

Traditional pulmonary function tests, such as spirometry and whole body plethysmography, are a critical aspect of the diagnosis, characterisation, and management of lung disease (Pellegrino et al., 2005). However, they require specific and often unfamiliar respiratory manoeuvres, and so are unsuitable for critically ill or sedated patients, and their reproducibility is reduced in both elderly and paediatric populations (Bellia et al., 2000, Beydon et al., 2007). The inspired sinewave technique (IST) is a novel method of measuring, or continuously monitoring, cardiopulmonary function which requires only passive patient cooperation (Clifton et al., 2013, Farmery, 2008, Phan et al., 2015). Therefore, the technology can provide simple, non-invasive cardiopulmonary measurements in both the outpatient and critical care settings.

Over successive tidal breaths the concentration of a tracer gas (e.g. nitrous oxide,  $N_2O$ ) is sinusoidally modulated in inspired air. The amplitude and phase of the expired sinewave are altered by the pulmonary ventilation and blood flow (if the tracer gas is soluble) and distorted further by ventilatory heterogeneity (VH). A mathematical model of the lung processes flow and concentration data and recovers values for cardio-respiratory variables such as end-expired lung volume (ELV), pulmonary blood flow ( $\dot{Q}_p$ ) and, in addition, indices for VH can be calculated.

The technique originated from the work of Zwart et al. (Zwart et al., 1978, Zwart et al., 1976) and was extended by Hahn et al. (Hahn et al., 1993, Hahn, 1996) by using patient safe gases such as  $O_2$  and low concentrations of  $N_2O$ , and developing a more realistic tidal ventilation lung model (Gavaghan and Hahn, 1996, Whiteley et al., 2001). Preliminary investigations in animals and human participants have since shown that the technique has the potential to

provide useful measures of cardiorespiratory function(Williams et al., 1998, Clifton et al., 2013, Farmery, 2008). In this investigation we firstly aimed to: 1) determine the accuracy of the IST measurement of ELV in healthy participants, by examining its agreement with FRC measured via whole body plethysmography ( $FRC_{pleth}$ ), and 2) examine the repeatability of the ELV measurement. Moreover, as healthy ageing results in several alterations in pulmonary structure and function – such as increases in lung compliance, the enlargement of airspaces and increases in VH(Turner et al., 1968, Gilleooly and Lamb, 1993, Verbanck et al., 2012) – this investigation also aimed to assess whether the IST estimates ELV and VH are age dependent.

## **MATERIALS AND METHODS**

### Ethical approval

All participants received verbal and written information regarding the protocol and experimental procedures before giving their written informed consent. The study's protocol was approved by an NHS ethical committee (16/SC/0057, ethics protocol 1.0) and conforms to the *Declaration of Helsinki, 2013*, except for registration in a database. 48 never-smoker participants volunteered for the study – characteristics and pulmonary function test data are shown in Table 1. The age of participating subjects ranged between 20-86, and all were defined as healthy through clinical screening with the following criteria: no childhood/past medical history of respiratory disease, no history of respiratory symptoms suggestive of disease, no upper respiratory tract infections in the previous 8 weeks, and no history of smoking.



<b>N</b>	48	
<b>Male/Female</b>	26/22	
	<b>Mean (SD)</b>	<b>% Pred (SD)</b>
<b>Age (years)</b>	53.5 (22)	-
<b>Height (m)</b>	1.7 (0.1)	-
<b>Weight (kg)</b>	71.5 (12)	-
<b>BMI (kg/m<sup>2</sup>)</b>	24.9 (4.2)	-
<b>FEV<sub>1</sub> (L)</b>	3.3 (1)	102.4 (17.1)
<b>FVC (L)</b>	4.3 (1.3)	105.6 (18.5)
<b>FEV<sub>1</sub>%FVC</b>	77.8 (6.9)	96.5 (7.5)
<b>TLC (L)</b>	6.3 (1.3)	105.4 (13.2)
<b>RV (L)</b>	2.2 (0.8)	104.4 (20.7)
<b>FRC (L)</b>	3.4 (0.8)	107.5 (16.6)
<b>T<sub>LCO</sub></b>	7.8 (2.6)	98.7 (15.5)
<b>K<sub>CO</sub></b>	1.5 (0.3)	94.6 (16)
<b>VA/TLC</b>	0.87 (0.05)	-
<b>ELV<sub>180</sub></b>	2.3 (0.8)	-
<b>ELV<sub>60</sub></b>	1.5 (0.5)	-
<b>Q<sub>P</sub></b>	4.9 (1.7)	-

Table 1. Participant characteristics and pulmonary function test measurements. SD, standard deviation; BMI, body mass index; FEV<sub>1</sub>, forced expiratory volume in one second; FVC, forced vital capacity; TLC, total lung capacity; residual volume; FRC, functional residual capacity; RV, residual volume; T<sub>LCO</sub>, transfer factor for carbon monoxide; K<sub>CO</sub>, transfer coefficient for carbon monoxide; VA/TLC, ratio between a single-breath helium dilution TLC measurement (VA) and TLC from whole body plethysmography; ELV<sub>180</sub> & ELV<sub>60</sub>, IST estimate of end-expired lung volume measured using a

sinewave with a 180 second or 60 second period respectively;  $Q_p$ , IST estimate of pulmonary blood flow. % predicted values obtained via reference values: Quanjer et al 1993, 2012.

## **Study protocol**

All procedures for each participant were performed on the same day within the Respiratory Medicine Department of the Churchill Hospital – Oxford University Hospitals NHS Foundation Trust. Prior to testing, a medical history and anthropometric data were recorded. Participants then underwent traditional pulmonary function testing: spirometry, body plethysmography and the single breath test of carbon monoxide uptake (Jaeger MasterScope Body, Carefusion); followed by the IST test.

## **IST test**

Participants were seated and asked to breathe quietly through a face mask connected to a mainstream infrared  $N_2O$  and  $CO_2$  sensor (SquareOne Technology) and an ultrasonic flowmeter (VenThor – 22/2A) – see figure 1 for a schematic representation of the set up. At the start of each inhalation a small quantity of  $N_2O$  was injected into the participants' inspired gas by a mass flow controller (Alicat Scientific, Inc., USA). The volume injected was proportional to each breath's inspiratory flow, and over successive breaths the concentration of inspired  $N_2O$  oscillates in a sinewave pattern around a set mean (4%) with a predetermined amplitude (3%) and frequency (60 sec or 180 sec period). The inspired  $N_2O$  fraction can therefore be defined by the following:

$$F_I(t) = \bar{F}_I + \Delta F_I \sin\left(\frac{2\pi}{T}t + \Phi\right) \quad \text{eq. 1}$$

where  $\bar{F}_I$  and  $\Delta F_I$  are the mean and amplitude of the N<sub>2</sub>O sinusoid respectively,  $T$  is the period, and  $\Phi$  is the phase.

In the absence of venous recirculation of the N<sub>2</sub>O sinewave, which is known to be negligible at the periods used in the current study, i.e. <5min, (Hahn et al., 1993, Gavaghan and Hahn, 1995) forcing sinusoidal inspired N<sub>2</sub>O concentrations results in the end-tidal (i.e. alveolar) N<sub>2</sub>O concentrations to also oscillate sinusoidally. Figure 2 shows a typical data set recorded from the IST test.

Using a single-compartment tidal ventilation model of the lung, the resulting amplitude and phase of the expired sinewave allows the estimation of  $\dot{Q}_P$  and alveolar volume ( $V_A$ ). The sum of  $V_A$  and airways dead space ( $V_D$ ), as measured via the Bohr technique (Phan et al., 2017), from the IST estimate of end-expired lung volume (ELV). The period (in seconds) of the inspired N<sub>2</sub>O sinewave used to estimate ELV is signified by the subscript – i.e. ELV<sub>180</sub> or ELV<sub>60</sub>. Modelling and preliminary empirical data has shown that ELV<sub>180</sub> has the closest agreement with FRC<sub>pleth</sub>. The lung model and estimation steps have been described elsewhere (Clifton et al., 2013, Phan et al., 2015, Gavaghan and Hahn, 1996), a summary of which is given in the appendix.

### **Indices of ventilation heterogeneity (VH)**

Three potential indices are proposed:

- 1) ELV<sub>180</sub>/FRC<sub>pleth</sub>
- 2) ELV<sub>180</sub>/FRC<sub>pred</sub>
- 3) ELV<sub>60</sub>/ELV<sub>180</sub>

where lower values suggest greater VH and higher values towards 1 suggest homogeneity.

The first two indices rely on the nature of *single compartment* models used to estimate lung volume (ELV). For the IST, ELV measurements in a uni-compartmental lung are a predictable function of ventilation, the sinewave period, and the attenuation of the expired sinewave as described by equation 1, and errors (underestimations) in the calculated volume suggests a degree of VH(Whiteley et al 2001). As such, increasing heterogeneity will result in greater disparity between  $ELV_{180}$  and  $FRC_{pleth}$ , or that estimated via prediction equations ( $FRC_{pred}$ ):

$$Male FRC_{pred} = 2.34 \times H + 0.01 \times A - 1.09$$

$$Female FRC_{pred} = 2.24 \times H + 0.001 \times A - 1$$

where H is standing height in metres and A is age in years(14)

The third index relies on ELV measurements becoming more dependent on sinewave period with increasing VH. In a homogeneous (uni-compartmental) lung the ELV measurement should be unaffected by the period used. However, preliminary modelling and experimentation has uncovered that where VH exists, estimated cardiopulmonary variables become period dependent(Whiteley et al., 2001). Therefore, the ratio of two ELV measurements made using different sinewave periods ( $ELV_{60}$  and  $ELV_{180}$ ) may estimate the degree of VH.

In addition, a further simple index (VA/TLC) of gas mixing inefficiency and VH has been calculated, which is the ratio between a single-breath helium dilution TLC measurement (VA) and a TLC measurement from whole body plethysmography (Cotes et al. 2006).

### **IST test protocol**

Participants were seated and rested for 5 minutes prior to each IST test. For the duration of the IST test participants are asked to breathe quietly through a face mask, sealed with no

leaks, held up in front of them by an adjustable articulating arm. All participants performed two IST tests – 3 minutes of inhaling a constant concentration of N<sub>2</sub>O (4%) followed by 4 minutes of forcing an inspired N<sub>2</sub>O sinewave (mean = 4%, amplitude = 3%) at periods of 180 sec and 60 sec. Participants then repeated these two tests after a 15 minute interval.

### **Statistical analysis**

All statistical analysis was conducted using Microsoft Excel (2016) and a standard statistical package (SPSS, Chicago, IL, USA). The agreement between FRC<sub>pleth</sub> and ELV<sub>180</sub> was assessed using Bland-Altman analysis. The repeatability of duplicate ELV<sub>180</sub> measurements were assessed using linear regression analysis, Bland-Altman analysis and the coefficient of variation (standard deviation/mean x 100). The first and second ELV<sub>180</sub> measurements were also compared using a student's paired t-test with statistical significance taken as p<0.05. Multiple stepwise regression analysis was performed on the three VH indices: ELV/FRC<sub>pleth</sub>, ELV/FRC<sub>pred</sub>, ELV<sub>60</sub>/ELV<sub>180</sub>, and independent variables included age, sex, height, and FRC<sub>pleth</sub>. Multiple stepwise regression analysis was also performed for ELV<sub>180</sub> and FRC<sub>pleth</sub>, and independent variables included age, sex and height. The statistical significance level for retention was set at p<0.05. When sex was uncovered as a significant factor for the ELV<sub>180</sub> and FRC<sub>pleth</sub> measurements, multiple regression analysis was repeated for each sex. Pearson's correlation coefficient was also calculated for the three IST indices of VH and pulmonary function test measurements: FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and VA/TLC.

## **RESULTS**

### **Indices of ventilation heterogeneity**

Figures 3A and 3B show that ELV<sub>180</sub>/FRC<sub>pleth</sub> and ELV<sub>180</sub>/FRC<sub>pred</sub> decrease as a function of age, suggesting an increase in VH. The other assessed independent variables (sex, height and

FRC<sub>pleth</sub>) did not reach statistical significance in the multiple regression analysis for all three heterogeneity indices, and although age was a significant contributor to the regressions of ELV<sub>180</sub>/FRC<sub>pleth</sub> and ELV<sub>180</sub>/FRC<sub>pred</sub> (p<0.05) it did not reach significance for ELV<sub>60</sub>/ELV<sub>180</sub> (figure 3C). Regression equations for these VH indices are shown in table 2, and their correlation with pulmonary function test measurements are shown in table 3.

	Regression equations	Adjusted R <sup>2</sup>
<b>VH indices</b>		
ELV <sub>180</sub> /FRC <sub>pleth</sub>	-0.0048 x A + 0.9427	0.62
ELV <sub>180</sub> /FRC <sub>pred</sub>	-0.0041 x A + 0.9478	0.28
ELV <sub>60</sub> /ELV <sub>180</sub>	-	-
<b>Lung volumes</b>		
ELV <sub>180</sub>		
Men	4.956 X H - 0.012 x A - 5.325	0.71
Women	4.292 X H - 0.007 x A - 4.794	0.79
FRC <sub>pleth</sub>		
Men	5.635 X H + 0.008 x A - 4.989	0.71
Women	4.989 X H + 0.013 x A - 5.928	0.69

Table 2. Regression equations for IST indices of VH (ELV<sub>180</sub>/FRC<sub>pleth</sub>, ELV<sub>180</sub>/FRC<sub>pred</sub>, ELV<sub>60</sub>/ELV<sub>180</sub>), and for ELV<sub>180</sub> and FRC<sub>pleth</sub>. H, Height (m); A, age(years).

	FEV <sub>1</sub>	FEV <sub>1</sub> /FVC	VA/TLC
<b>VH Indices</b>			
ELV <sub>180</sub> /FRC <sub>pleth</sub>	0.63*	0.34*	0.56*
ELV <sub>180</sub> /FRC <sub>pred</sub>	0.64*	0.15	0.57*
ELV <sub>60</sub> /ELV <sub>180</sub>	0.08	-0.02	0.04

Table 3. Pearson's correlation coefficients (r) for IST indices of VH (ELV<sub>180</sub>/FRC<sub>pleth</sub>, ELV<sub>180</sub>/FRC<sub>pred</sub>, ELV<sub>60</sub>/ELV<sub>180</sub>), with FEV<sub>1</sub>, FEV<sub>1</sub>/FVC and VA/TLC. \*, p<0.05).

### **Lung volume (ELV<sub>180</sub>): repeatability and agreement with FRC<sub>pleth</sub>**

The mean measured ELV<sub>180</sub> was 2.31 litres ( $\pm 0.75$ ; range: 1.01-3.55). Bland-Altman analysis (figure 4) shows that the mean differences between repeated measurements was -0.05 litres ( $\pm 0.2$ ), 1.29% of the mean, and the 95% limits of agreement were between -0.42 and 0.31 litres. The coefficient of variation for repeated ELV<sub>180</sub> measurements was 4.8%. Linear regression analysis between these measurements produced an  $R^2$  of 0.94,  $y = 0.983 + 0.0871x$  (figure 5). The first and second measurement were not statistically different ( $p = 0.76$ ).

The mean measured FRC<sub>pleth</sub> was 3.4 litres (0.85; range 2.11-5.19), with an average percent predicted value of 107.5% (16.6; range 67.4-120). Bland-Altman analysis (figure 6) shows that the mean differences between FRC<sub>pleth</sub> and ELV<sub>180</sub> measurements was -1.08 litres (0.53), and the 95% limits of agreement were between -2.1 and -0.37 litres. Regression equations for ELV<sub>180</sub> and FRC<sub>pleth</sub> from the current cohort of participants are shown in table 2. An age-normalised Bland-Altman plot is shown in figure 7.

## **DISCUSSION**

### **Lung volume**

If we accept FRC<sub>pleth</sub> as the gold standard measure of end-expiratory lung volume, the current study suggests that the IST test underestimates lung volume by approximately 1.09L. Underestimations are expected as body plethysmography measures the volume of compressible gas in the thorax and inevitably includes volumes that are either located in regions that are poorly ventilated or that do not communicate with airways – including that within the abdominal cavity. This volume cannot be quantified by the IST test, or traditional dilution tests (Schaanning and Gulsvik, 1973), and so contributes to an inevitable bias.

However, the underestimation of  $ELV_{180}$  relative to  $FRC_{pleth}$  clearly has a component of age dependency. Multivariate linear regression analysis on the current cohort uncovered that  $FRC_{pleth}$  increases by approximately 0.01L per year of age and, interesting,  $ELV$  decreases by approximately -0.01L per year (Table 2). This implies that the accuracy of the IST estimate of lung volume reduces with age (and likely reflects increasing  $VH$  – see Ageing and Ventilatory Heterogeneity section). However, the agreement of  $ELV_{180}$  with  $FRC_{pleth}$  can be improved. Using the regression equations from the multivariate analysis,  $ELV_{180}$  and  $FRC_{pleth}$  can be normalised to that predicted of a 20-year-old for a given height and sex. Bland-Altman analysis of age-normalised  $ELV_{180}$  and  $FRC_{pleth}$  (figure 7) shows a much smaller mean bias of -0.35L (with 95% limits of agreement at  $\pm 0.73L$ ). This underestimation of  $ELV_{180}$  is similar to that of helium dilution measurement of  $FRC_{pleth}$  (e.g. -0.3L; Schaanning and Gulsvik, 1973). As such, simply normalising the  $ELV_{180}$  measurement for age provides a more accurate measurement of end-expiratory lung volume.

The  $ELV$  measurement was very repeatable between tests separated by a 15 minute period, with a coefficient of variation of 4.77% - similar that of whole body plethysmography and helium dilution measurements of end-expired lung volume, which range from approximately 4-7% and 5-10% respectively (Hankinson et al., 1998). There was no effect of test order on the duplicated measurements as shown by Bland-Altman analysis of the first and second  $ELV$  measurements (mean bias of 0.05L, 95% limits of agreement of 0.36), and the failure of their means to reach statistically significant difference. The mean absolute percentage difference between the duplicated measurements was 7.3% ( $\pm 7.6$ ). This complies with the current standard guidelines for acceptable differences between duplicated helium dilution and nitrogen washout measurements of lung volumes (Wanger et al., 2005) (10%).

Accurate and precise measurements of lung volume offer valuable information to respiratory clinicians, as alterations in lung mechanics occur in several pathologies (Pride and Macklem,



2011). In addition, the IST test might provide clinically useful information in the critical care setting; Continuous monitoring of ELV could be of great value in ventilated patients where positive end expiratory pressures have been applied, by reducing the risk of ventilator induced lung injury via volutrauma or atelectrauma (Slutsky and Ranieri 2013). #

However, whilst volume estimations are based upon a single compartment lung model, significant respiratory disease and VH will likely impair the accuracy of the technique. Indeed, multi-compartmental lung models could be used to provide better estimates of lung volume where VH exist (Whiteley et al. 2001), and further work in patients is required to examine this. However, an advantage of using uni-compartment models is that they can be more easily ‘inverted’ – where by recorded physiological data (e.g. the attenuation of the expired sinewave) can be inserted into them and recover estimations of cardiorespiratory parameters (e.g. ELV or  $\dot{Q}_P$ ; Hahn & Farmery, 2003). Indeed, finding inverse solutions for these variables in the heterogeneous lung can be problematic when using more complex and mathematically flexible multi-compartment models (Whiteley et al. 2001). In addition, the physiological interpretation of such models can be challenging for physiologists and clinicians. Altogether, the practicality and value of using single compartment models have ensured their continued use (Farmery, 2008; Hahn & Farmery 2003).

### **Ageing and Ventilatory Heterogeneity**

It is well established that ageing results in several changes in pulmonary structure and function, including an enlargement of airspaces and increases in airflow obstruction (Janssens et al., 1999), both of which likely contribute to greater degrees of VH. Single-breath and multi-breath nitrogen washout (SBNW and MBNW) tests assess the efficiency of ventilation distribution, where greater VH is reflected in steeper phase III slopes and delayed N<sub>2</sub>

clearance respectively(Robinson et al., 2013). With advancing age, phase III of N<sub>2</sub> expirograms become steeper(Sandqvist and Kjellmer, 1960, Roberts et al., 1991), and the clearance of N<sub>2</sub> during a MBNW test becomes delayed – as demonstrated by the positive linear associations between age and LCI, S<sub>cond</sub> and S<sub>acin</sub>(Verbanck et al., 2012).

The degree of disparity between the IST measure of lung volume (ELV<sub>180</sub>) and the *true lung* volume, might similarly provide a useful index of VH. This is because ELV measurements are a perfectly predictable function of ventilation, the sinewave period, and its attenuation, and errors in the calculated volume suggests a degree of VH(Whiteley et al 2001). This is comparable to a multi-breath nitrogen (N<sub>2</sub>) washout test, where deviations in the N<sub>2</sub> washout curve from a perfectly exponential decay suggests a degree of VH. As such, the findings of the current study also suggest that healthy ageing is associated with increases in VH, as decreases in the ratio of ELV<sub>180</sub> to FRC<sub>pleth</sub> and ELV<sub>180</sub> to FRC<sub>pred</sub> with advancing age were observed. Indeed, there was a significant positive correlation between these IST indices of VH and VA/TLC, a simple measure of gas mixing inefficiency (Hansen, 2011), and also with measures of airflow obstruction (FEV<sub>1</sub> and FEV<sub>1</sub>/FVC; table 3). The linear decrease in ELV<sub>180</sub>/FRC<sub>pleth</sub> occurred within the age range 20 to 85 years, and between these ages it declined by approximately 38% (an average of 0.58% per year). ELV<sub>180</sub>/FRC<sub>pred</sub> also decreased linearly and between the ages of 20 and 85, where it reduced by approximately 31% (an average of 0.48% per year). These changes in ELV<sub>180</sub>/FRC<sub>pleth</sub> and ELV<sub>180</sub>/FRC<sub>pred</sub> with advancing age are comparable to extrapolated increases in LCI, S<sub>cond</sub> and S<sub>acin</sub>, which increase by 0.3-0.7% per year between the ages 20-85 – assuming continued linearity(Verbanck et al., 2012).

The pulmonary changes associated with ageing have been collectively termed ‘senile-emphysema’ (Knudson, 1991, Janssens et al 1999). However, there are clear histological distinctions with *true* emphysema which also results in the destruction of alveolar walls and a

more heterogenous increases in airspace size suggesting significantly greater degrees of VH (Verbeken et al., 1992). Therefore, methods that quantify VH, such as the IST test, have potential as a clinically useful tool in the diagnosis and/or staging of chronic obstructive pulmonary disease; and further work in patients is required to examine this possibility. However, our findings from the current investigation suggest that the physiological increases in VH with healthy ageing must be accounted for in any clinical investigation.

We found no change in third IST index of VH ( $ELV_{60}/ELV_{180}$ ) with age. Given the evidence that ageing can increase VH (Verbanck et al., 2012) and that our other indexes ( $ELV_{180}/FRC_{pleth}$ ,  $ELV_{180}/FRC_{pred}$  and  $VA/TLC$ ) demonstrate this occurring in the current group of participants, it is likely that this index has failed to detect existing differences in VH between participants. This could be a consequence of using an unsuitable pair of sinewave periods, or this index might not be sensitive enough to detect small changes in VH associated with age. This can be examined further by using the index to compare healthy participants with patients with obstructive lung diseases, a group known to have significantly increased VH (Aurora et al., 2004, Verbanck et al., 1998).

## **Summary**

The IST test is a novel, non-invasive method of estimating cardiopulmonary function that requires only passive patient cooperation. The current study has demonstrated that the IST indices of VH,  $ELV_{180}/FRC_{pleth}$  and  $ELV_{180}/FRC_{pred}$ , are sensitive to age. The IST test's estimation of end expired lung volume ( $ELV_{180}$ ) was very repeatable, and has close agreement with  $FRC_{pleth}$  when normalised for the effects of age. Altogether, the IST test has the potential to provide clinically useful information, and so further work with mechanically ventilated patients, and those with obstructive lung disease is warranted.

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## APPENDIX: SUMMARY OF THE INSPIRED SINEWAVE TECHNIQUE

Figure A1 Illustrates the single compartment model of the cardiopulmonary system. The lung consists of one dead space compartment  $V_D$ , and one lung compartment  $V_A(t)$  that expands and contracts with tidal volume. The body has one compartment  $V_{body}$ , with  $\dot{Q}_P$  flowing through the lung for gas exchange.

When a tracer gas (e.g.  $N_2O$ ) is inhaled, it passes through the dead space into the lung. It then diffuses into arterial blood, and distributes to different parts of the body. After some transit time, the tracer gas travels back to the lung in mixed venous blood. Some will cross the blood-gas barrier into the lung, before eventually being exhaled in expired gas.

During an IST test, over successive breaths the inspired concentration of the tracer gas  $F_I(t)$  follows a sinewave pattern. Consequently, after a short transient time the alveolar ( $F_A(t)$ ), arterial ( $F_a(t)$ ) and mixed venous ( $F_{\bar{v}}(t)$ ) concentrations will all follow sinewave patterns.

The body compartment acts as a low-pass filter to diminish the amplitude of the mixed venous sinewave  $F_{\bar{v}}(t)$ . When the sinewave period is short enough ( $\leq 5$ mins), the amplitude of  $F_{\bar{v}}(t)$  is small enough to assume it is constant, and therefore  $F_{\bar{v}}(t) = F_I^0$  in which  $F_I^0$  is the mean of inspired sinewave  $F_I(t)$ .

The mass balance of the lung compartment for breath  $n^{\text{th}}$  therefore can be written as:

$$F_{E,n-1} \times V_A + F_{I,n} \times (V_{T,n} - V_D) + F_{E,n-1} \times V_D - \lambda \times \dot{Q}_P \times (F_{E,n} - F_{\bar{v}}) \times \Delta t_n = V_A \times F_{E,n} + V_{T,n} \times F_{E,n}$$

$$\Leftrightarrow V_A \times (F_{E,n} - F_{E,n-1}) + \lambda \times \dot{Q}_P \times (F_{E,n} - F_I^0) \times \Delta t_n = V_D \times (F_{E,n-1} - F_{I,n}) + V_{T,n} \times (F_{I,n} - F_{E,n})$$

in which:

$V_A$ : the alveolar lung volume;

$\dot{Q}_P$ : the pulmonary blood flow;

$V_D$ : the deadspace, estimated from the Bohr method;

$F_{\bar{I},n}$ : the mean inspired concentration of breath nth;

$F_{E,n-1}, F_{E,n}$ : the end expired concentrations of breath (n-1) and n;

$\lambda$ : the solubility of N<sub>2</sub>O, 0.47;

$F_{\bar{v}}$ : the mixed venous concentration, assumed to be equal to the mean of the inspired sinewave concentration at steady state  $F_I^0$ ;

$\Delta t_n$ : the duration of breath n<sup>th</sup>;

$V_{T,n}$ : the tidal volume of breath n<sup>th</sup>;

First,  $V_D$  is estimated using the Bohr method applied to an N<sub>2</sub>O signal:

$$V_D = V_T \frac{F_E - F_{\bar{E}}}{F_E - F_{\bar{I}}}$$

in which  $F_{\bar{E}}$  is the mean expired concentration. To compensate for the error associated with the non-uniform inspired concentration, a modified Bohr method has been proposed improving the accuracy of the airway deadspace estimation(Phan et al., 2017). For a series of breaths, a set of linear equations can be established from eq (2) and solved for  $V_A$  and  $\dot{Q}_P$ . ELV can then be calculated as sum of the  $V_D$  and  $V_A$ . Theoretically, three consecutive breaths would be sufficient to construct the linear equations to find solutions for  $V_A$  and  $\dot{Q}_P$ . In practice, the use of all breaths within a complete sinewave period is recommended to improve repeatability. Interested readers can refer elsewhere(Phan et al., 2015) for further detail.

## FIGURES

Figure 1. Schematic representation of the Inspired Sinewave Device. The participant inhales and exhales air through flow meter (FM) and gas sensor (GS). At the start of each inspiration a small quantity of  $N_2O$  is delivered into the participants inspired gas via a mass flow controller (MFC). A mathematical model of the lung processes flow and concentration data and recovers values for cardio-respiratory variables such as dead space, alveolar volume, pulmonary blood flow, and, in addition, an index for ventilation heterogeneity can be calculated.

Figure 2. A typical data set collected from one participant during a 4 minute IST test. The green line is the expired  $N_2O$  concentration measured by the mainstream infrared gas sensor. The blue and red crosses are the  $N_2O$  concentrations in inspired gas and end-tidal gas respectively. The blue and red lines are the inspired and expired  $N_2O$  sinewaves respectively.

Figure 3A-C. Scatterplot of  $ELV_{180}/FRC_{pleth}$ ,  $ELV_{180}/FRC_{pred}$ , and  $ELV_{60}/ELV_{180}$  versus age with a linear regression line.

Figure 4. Bland-Altman plot showing the agreement of two repeated IST measurements of ELV ( $ELV_{180(1)}$  vs  $ELV_{180(2)}$ ). LOA, limits of agreement.

Figure 5. Linear regression analysis of the two repeated IST measurements of  $ELV_{180}$  ( $ELV_{180(1)}$  vs.  $ELV_{180(2)}$ ).

Figure 6. Bland-Altman plot showing the agreement between  $ELV_{180}$  and  $FRC_{pleth}$ . LOA, limits of agreement.

Figure 7. Bland-Altman plot showing the agreement between age-normalised  $ELV_{180}$  and  $FRC_{pleth}$ . Using the regression equations from multivariate analysis,  $ELV_{180}$  and  $FRC_{pleth}$  are

normalised to that predicted of a 20 year old for a given height and sex. LOA, limits of agreement.

Figure A.1: The single compartment model of the lung and circulatory system

## **AUTHORS CONTRIBUTIONS**

Experiments were performed within the Respiratory Medicine Department of the Churchill Hospital – Oxford University Hospitals NHS Foundation Trust. RMB, PP, NR, AF contributed to the conception and design of the work; RMB, PP, EP, AF contributed to the acquisition, analysis and interpretation of the data; RMB, PP, EP, NR, AF contributed to the drafting of the work and/or revising it critically. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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## Gas Delivery system

LabVIEW  
Controller

MFC

FM

GS

Participant

LabVIEW data  
acquisition

MATLAB  
analysis

Dead space

Lung Volume

Pulmonary blood flow

## Data capture/analysis















